Miklos Bodanszky

Principles of Peptide Synthesis

Second, Revised Edition

Springer-Verlag
Berlin Heidelberg New York London Paris
Tokyo Hong Kong Barcelona Budapest

Professor Dr. MIKLOS BODANSZKY
One Markham Road
Princeton, NJ 08540, USA

The first edition was published as Vol. 16 of the series
"Reactivity and Structure" (3-540-12395-4)

ISBN 3-540-56431-4 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-56431-4 Springer-Verlag New York Berlin Heidelberg

Library of Congress Cataloging-in-Publication Data. Bodanszky, Miklos. Principles of Peptide Synthesis / Miklos Bodanszky. – 2nd rev. ed. p. cm. – (Springer laboratory) Includes bibliographical references and index. ISBN 3-540-56431-4 (Berlin: acid-free paper). – ISBN 0-387-56431-4 (New York: acid-free paper) 1. Peptides – Synthesis. I. Title. II. Series. QP552.P4B63 1993 547.7'56–dc20 93-3332

Printed in Germany

Typesetting: Best-set Typesetters Ltd, Hong Kong
51/3145-5 4 3 2 1 0 – Printed on acid-free paper

Preface to the Second Edition

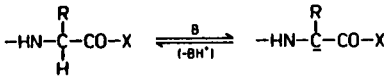
The attempt to render PRINCIPLES OF PEPTIDE SYNTHESIS somewhat resistant to the passing of time could, of course, be only partially successful. In the decade that has elapsed since the completion of the manuscript, the discovery of a long series of biologically active peptides together with the major application[redacted] of peptide hormones, such as calcitonin, the blood-pressure-lo[redacted] enzyme inhibitor, the pseudopeptide captopril, in medicine, and the large-scale production of the sweetener, aspartame, have given new impetus to peptide chemistry. A considerably widening of interest in peptide synthesis, both in academia and in industry, ensued and numerous novel methods appeared in the literature. It seemed timely to update the original version of PRINCIPLES OF PEPTIDE SYNTHESIS.

Preparation of this Second Edition provided a welcome opportunity for revising the text. This revision went beyond the correction of printer's errors and other mistakes. A more substantial modification of the first edition was prompted by a thorough critique by Professor G.T. Young of Oxford University. I considered his recommendations carefully and adopted most of them. Some changes in the evaluation of methods have also been made. For instance I reexamined the principle of coupling reagents and introduced the concept of "true coupling reagent".

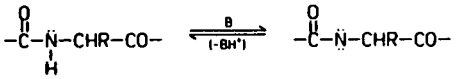
Only a part of the new procedures could be fitted into the appropriate chapters of the first edition, hence most of th[redacted]erial published between 1982 and 1992 was assembled in the co[redacted]ding Chapter VIII. This separation of old and new served not merely convenience but also allowed me to attempt an assessment of new ideas and to discern novel trends.

Princeton, New Jersey, 1993 MIKLOS BODANSZKY

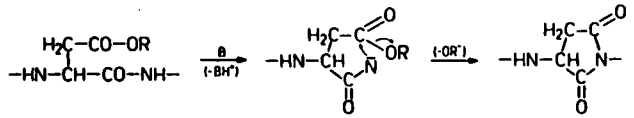
In esters and in various activated derivatives of acylamino acids, no such obstacle exists against proton abstraction. In fact the electron withdrawing forces present in the activating group "X" enhance the activity of the α-hydrogen and facilitate its abstraction:



An obvious consequence of carbanion formation is the partial or total loss of chiral purity. Proton abstraction might be reversible and the equilibrium of the reaction might lie far to the left: gradually more and more molecules will pass through a carbanion stage and suffer irreparable racemization. Therefore, the risk of racemization is inherent in peptide synthesis and in order to avoid it, it must be carefully considered. There are, however, side reactions in which proton abstraction occurs not at the α-carbon atom but at the amide nitrogen of an acylamino acid. The additional



unshared pair of electrons on the nitrogen atom renders the latter, in spite of the presence of the carbonyl substituent, a good nucleophile. Thus, it can participate in numerous side reactions, particularly in intramolecular attacks resulting in cyclizations. For instance, the formation of succinimide derivatives is usually preceded by proton abstraction from the amide nitrogen of an aspartyl amino acid residue:



Analogous cyclization reactions and *O*-acylations initiated by proton abstraction will be discussed in separate sections.

1.1 Racemization

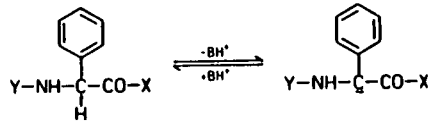
1.1.1 Mechanisms of Racemization

Understanding the mechanisms of racemization seems to be necessary for its prevention. Accordingly, a considerable amount of experimental work has been carried out in this area, and was skillfully rendered in a review article by Kemp [4]. At this place we confine the discussion to the

principal processes of base catalyzed racemization of activated acylamino acids. Three distinct pathways can be recognized:

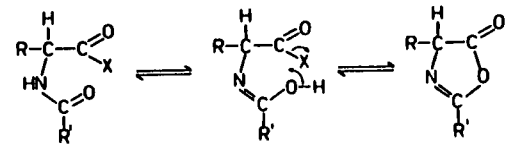
a) direct abstraction of the α-proton,
b) racemization via reversible β-elimination and
c) racemization through azlactones [5(4H)-oxazolones].

The simple proton abstraction mechanism might be a contributor in several processes but it is the dominant pathway only in very special cases such as the rapid racemization of derivatives of phenylglycine, an amino acid which is not a constituent of proteins although it occurs in microbial peptides:

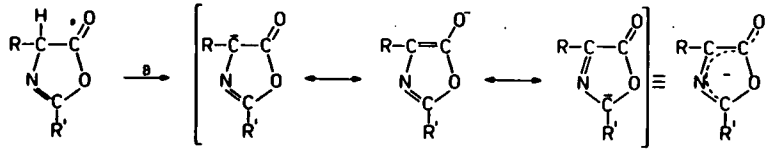


(where Y is a protecting group and X an activating group). The conspicuous racemization of active esters of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine [5] was usually explained by the reversible, base-induced elimination of benzylmercaptane and this explanation was supported by the fortuitous isolation of *N*-benzyloxycarbonyl-*S*-benzyl-DL-cysteine thiobenzylester from a solution of the *p*-nitrophenyl ester containing triethylamine. Subsequent studies, carried out e.g. with radioactively labelled benzylmercaptane [6], demonstrated that racemization of reactive cysteine derivatives can proceed without the elimination of the thiol. Further examination of the problem led to a proposal [7] in which a direct interaction between the chiral carbon-atom and the sulfur atom, involving the *d*-orbitals of the latter, is invoked. The problem, however, is further complicated by the often observed racemization of reactive derivatives of *O*-benzyl-serine (in which clearly no *d*-orbitals are present) during coupling. Thus the *d*-orbitals of the sulfur atom might contribute to but cannot be solely responsible for the racemization of *S*-alkyl-cysteine. A rationale, applicable both for cysteine and for serine derivatives, is enol-stabilization by intramolecular hydrogen bonds, with the sulfur or the oxygen atom, respectively, as bridgeheads. The ready racemization noted in reactive derivatives of β-cyano-alanine [8] can be explained simply by the strong electron-withdrawing effect of the cyano group.

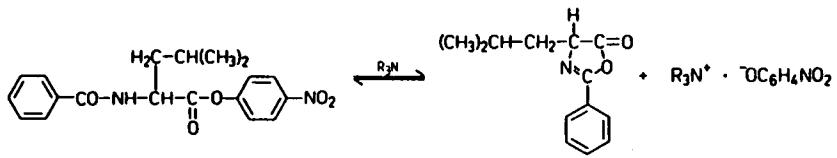
The best studied and probably most important mechanism of racemization involves the formation of azlactones [9]:



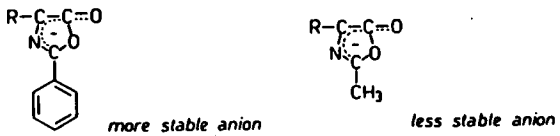
The explanation for the tendency to racemization of azlactones lies in the ease by which the acidic proton can be abstracted by bases from the chiral center due to resonance stabilization of the carbanion generated in the process:



Azlactones are good acylating agents and could be useful for the activation of the carboxyl component. Yet, delocalization of the negative charge in the deprotonated intermediate provides them with sufficient lifetime to endanger the chiral purity of the product. The formation of an azlactone could be demonstrated [10] by its characteristic carbonyl frequency ($1832\,cm^{-1}$) when benzoyl-L-leucine *p*-nitrophenyl ester was exposed to the action of tertiary amines



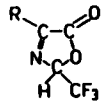
and equally convincing evidence incriminating the azlactone intermediate was found in the production of partially racemized benzoyl-leucyl-glycine ethyl ester when the reaction was completed with acylation of glycine ethyl ester. Characteristically, the unreacted portion of benzoyl-L-leucine *p*-nitrophenyl ester was recovered enantiomerically pure. Racemization through azlactone intermediates is influenced by several factors such as the nature of the amino acid involved, the solvent used in the reaction or the presence (or absence) of tertiary amines. The acyl group on the amine nitrogen, however, plays a decisive role in the conservation or loss of chiral purity. For instance, under identical conditions, benzoylamino acids are more extensively racemized than acetylamino acids [11]. Such differences seem to be related to the electronic forces operating in the acyl group. Beyond the formation of azlactones the *N*-acyl substituents of the oxazolinone can also affect the acidity of the hydrogen atom on the chiral center. Expressed in another way: the stability of the anion produced in proton abstraction by bases is enhanced by electron withdrawing effects in the acyl group:



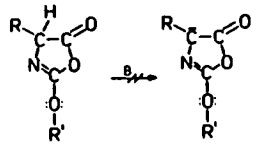
more stable anion *less stable anion*

It is probably not so much the formation of azlactones that is of primary importance in determining the rate of racemization but rather the electronic effects of the substituents of the oxazolinone, including those in the *N*-acyl group. Azlactones can be obtained in optically active form [12], and if immediately trapped by good nucleophiles [13], they can yield optically active products.

The influence of the *N*-acyl group on the stability of the anion generated through proton abstraction from the oxazolinone can range from extreme stabilization found in the formyl and trifluoroacetyl groups to pronounced destabilization shown by the benzyloxycarbonyl, *tert*-butyloxycarbonyl and other alkoxycarbonyl groups. In fact, trifluoroacetylamino acids yield an isomer [14] of the more common azlactones, an isomer in which the α-carbon atom is not chiral:



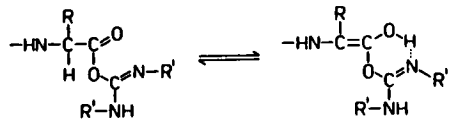
Until recently it was generally assumed that benzyloxycarbonylamino acids and, in general, amino acids protected by a urethane-type blocking group do not produce azlactones and hence are resistant to racemization during activation and coupling. Isolation [15] of optically pure oxazol-(4H)-ones, e.g. from the reaction of *tert*-butyloxycarbonyl-L-valine with water soluble carbodiimides contradicts such assumptions and suggests that the beneficial effect of urethane type protecting groups rests on the electron release provided by them and on the ensuing destabilization of the anion which could form by proton abstraction:



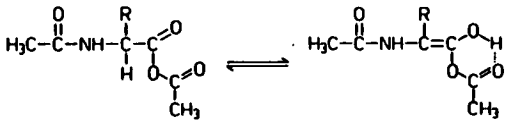
The chiral stability of proline derivatives was usually explained by the absence of an amide hydrogen in the *N*-acyl derivatives of this secondary amine. It appeared plausible that without such an amide hydrogen no azlactone should form. This explanation, however, ignores the possible

formation of protonated azlactones (oxazolonium salts). It was completely refuted by the ready racemization of *N*-methylamino acids [16] during activation and coupling. Thus, the chiral stability of proline is due to its rigid geometry rather than the fact that it is a secondary amine. Under certain conditions, e.g. in diketopiperazines, proline is readily racemized.

The role of bases in at least some of the racemization processes is beyond doubt. For instance, time and again the advantage of free amines over a mixture of amine salts with tertiary bases was noted. Less attention has been paid so far to the possibility of *intramolecular* base catalysis, although in several coupling methods the reactive intermediate contains a basic center and the latter could abstract the hydrogen from the chiral carbon atom. Since *O*-alkyl isoureas have pronounced basic character, it may not be farfetched to assume intramolecular proton abstraction by a basic nitrogen atom in the *O*-acyl-isourea intermediates of carbodiimide mediated coupling reactions. For instance hydrogen bond stabilized enols might play a role in such processes



which would then be analogous to the effect of excess acetic anhydride on optically active amino acids. Here racemization probably proceeds through enolization of mixed anhydrides:



1.1.2 Models for the Study of Racemization

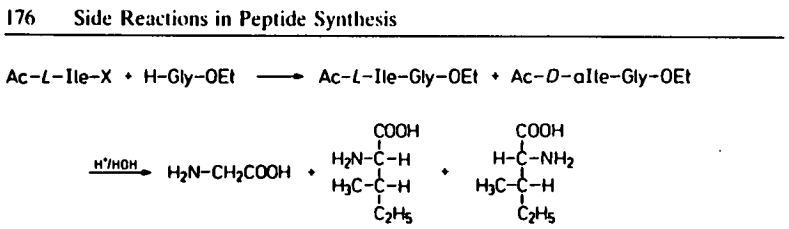
Numerous model systems have been proposed for the study of racemization. These systems are used to evaluate the effect of solvents, presence or absence of bases, temperature and other variables and last, but not least, the ability of different coupling methods to produce peptides without loss of chiral purity. The earliest suggestions came from Young's laboratory [17, 18] and involve the coupling of acetyl or benzoyl-L-leucine to glycine ethyl ester, followed by the examination of the optical rotation of the crude product. The results can be further refined by fractional crystallization

and analysis of the fractions by weight, optical rotation and melting point. The benzoyl group enhances the tendency for racemization, hence activation and coupling of benzoyl-L-leucine is a very sensitive racemization test.

A simple, and therefore frequently applied, model experiment was designed by Anderson and Callahan [19]. It involves the coupling of benzyloxycarbonylglycyl-L-phenylalanine to glycine ethyl ester. If racemization occurs in the process the product contains benzyloxycarbonylglycyl-DL-phenylalanyl-glycine ethyl ester, which is fairly insoluble in aqueous ethanol and can thus be separated and weighed. A word of caution is indicated here. This simple and useful method is reliable only if no by-products, other than the racemate, are formed in significant amount in the coupling reaction. Otherwise crystallization of the racemate might be impeded by the impurities and from the lack of crystallization the wrong conclusion, that there was no racemization, can be drawn. In principle, models should be so designed that the products of the test-experiment are not racemates but diastereoisomers and the conclusions are not based on negative evidence.

A more reliable, albeit also more time consuming, experiment is based on the coupling of benzyloxycarbonylglycyl-L-alanine to L-phenylalanylglycine ethyl ester (the "Kenner model") [20]. The diasteroisomers formed in the reaction are separated by countercurrent distribution. Somewhat less laborious are the methods introduced by Weygand and his associates [14, 21, 22], who condensed trifluoroacetyl-L-valine with L-valine methyl ester, or benzyloxycarbonyl-L-leucyl-L-phenylalanine with L-valine *tert*-butyl ester or trifluoroacetyl-L-prolyl-L-valine with L-proline methyl ester. The reaction products are examined with the help of vapor phase chromatography for the presence of diastereoisomers formed by racemization.

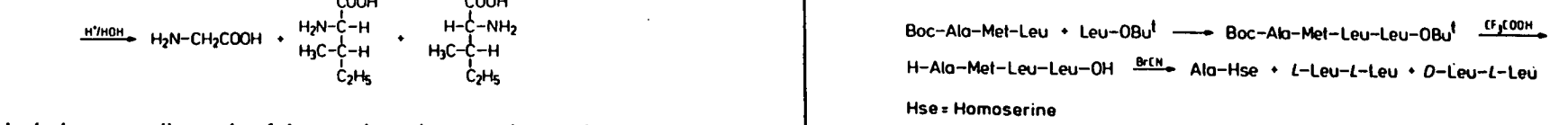
The test systems discussed so far are based on differences with respect to solubility or partition coefficient between diastereoisomers (or in the Anderson-Callahan test, between the racemate and the enantiomerically pure peptide derivative). An experimentally simple realization of the same principle is the examination of the products of model reactions by paper chromatography or thin layer chromatography [23]. Improvements in the realiability of the tests are also possible, e.g. the Young test can be perfected by the chromatographic separation of the products [24]. A more substantial simplification is, however, the use of the ubiquitous amino acid analyzer for the separation and quantative determination of the diasterosimers generated in the racemization tests. For instance coupling of acetyl-L-isoleucine [25] to glycine ethyl ester yields, in addition to the desired acetyl-L-isoleucylglycine ester, also acetyl-D-*allo*isoleucylglycine ethyl ester, if racemization occured in the reaction. Since alloisoleucine and isoleucine are routinely separated by the Spackman-Stein-Moore method [26], it is sufficient to



hydrolyze a small sample of the reaction mixture and to apply the hydrolysate to the analyzer. The main advantage of this model experiment is that no isolation of products is needed. This means a certain saving of time and effort, but more importantly the examination of the *crude* material assures that no distortion takes place in the isolation or separation of the products, thus no isomer is left in mother liquors, etc. The acetyl group has no major effect on the racemization of the amino acid to which it is attached, thus in this respect it can represent a peptide chain. This model can be applied for the study of the effect of coupling methods, solvents, tertiary amines added and also of the influence of the amino component, since glycine ethyl ester can be replaced by other nucleophiles. Yet, a certain limitation is caused, by the choice of isoleucine as the activated residue. It is a hindered amino acid and might suffer more loss in chiral purity than other less hindered residues which do not reduce the rate of the desired reaction and therefore allow less time for unimolecular processes such as racemization.

The same principle, separation of diasteroisomers on the amino acid analyzer, appears also in the "Izumiya test" [27, 28] in which a benzyloxycarbonylglycyl amino acid is coupled to an optically active amino acid benzyl ester and the products examined after deprotection by hydrogenation. This model system allows variations with respect to the amino acid residue which is exposed to racemizing conditions. Thus, instead of Z-Gly-L-Ala one can couple Z-Gly-L-Phe, etc. to L-Leu-OBzl and the nucleophile can also be so selected that detection of the diastereoisomers causes no difficulty. The contributions of Benoiton and his associates [29, 30], who used N^{ε}-benzyloxycarbonyl-L-lysine benzyl ester for amino component, lie in the same direction. The degree of racemization can be estimated, without deprotection and separation, through the examination of the nmr spectra of the coupling products. The model compounds acetyl-L-alanyl-L-phenylalanine methyl ester and acetyl-L-phenylalanyl-L-alanine methyl ester [31] allow the determination of the D-amino acid containing isomers by integration of the areas of the methyl protons of alanine while coupling of benzoylamino acids to N^{ε}-benzyloxycarbonyl-L-lysine methyl ester [32] permits a similar assessment of racemization through the examination of the methyl protons of the methyl ester group. In an interesting proposal [33] coupling of *tert*-butyloxycarbonyl-L-alanyl-L-methionyl-L-leucine to the *tert* butyl ester of L-leucine is followed by acidolysis and then by a treatment with cyanogen bromide in aqueous

acetic acid and by determination of the ratio of the two diastereoisomers, L-Leu-L-Leu and D-Leu-L-Leu with the help of the amino acid analyzer:



Hse = Homoserine

In a sophisticated and also very sensitive model experiment [34] benzyloxycarbonyl-L-alanyl-D-alanine is activated by the method to be tested and coupled to L-alanyl-L-alanine p-nitrobenzyl ester. The crude product is deblocked by hydrogenation and the mixture of the two isomeric tetrapeptides L-Ala-D-Ala-L-Ala-L-Ala and L-Ala-L-Ala-L-Ala-L-Ala, is exposed to the action of leucine aminopeptidase. The enzyme will catalyze the complete hydrolysis of the all-L peptide, the product of racemization, but leaves the peptide in which the second position is occupied by a residue with D-configuration intact. With respect to sensitivity this method is surpassed by the isotope dilution techniques introduced into peptide chemistry by Kemp and his coworkers [35–37]. Radioactively labeled benzyloxycarbonylglycyl-L-leucine or benzoyl-L-leucine is coupled to glycine ethyl ester followed by dilution with "cold" racemate and fractional crystallization until products with constant count per mg are obtained. This yields reliable information on racemization and allows the detection of very slight racemization which would be left unnoticed in the original versions of the Anderson-Callahan or the Young tests (cf. above).

Some problems, e.g. the base catalyzed racemization of active esters of protected amino acids or peptides can be investigated simply by following the change of optical rotation with time [38]. The effect of solvents, protecting groups, temperature, activating groups, etc. can be studied in this simple manner. With selected model compounds [39] it was possible to determine the scope and limitations of hindered amines in preventing racemization.

1.1.3 Detection of Racemization (Examination of Synthetic Peptides for the Presence of Unwanted Diastereoisomers)

Racemization during the activation and coupling of suitably protected amino acids occurs rarely but cannot be excluded. It is even more likely to occur in the activation and coupling of protected peptides. Therefore, it is desirable and sometimes absolutely necessary to examine the synthetic products for the presence of unwanted diastereoisomers. Such contaminants, if they are only minor constituents in the crude synthetic material, might be lost in the isolation process or during purification but can also accompany the principal product through these steps. A simple and

practical approach to the detection of diastereoisomers was devised by Manning and Moore [40]. A sample of the peptide is completely hydrolyzed with constant boiling hydrochloric acid and the mixture of liberated amino acids is acylated with an enantiomerically pure protected and activated amino acid, e.g. with L-leucine *N*-carboxy-anhydride. The resulting mixture of dipeptides is applied to the column of an automatic amino acid analyzer [26] which can separate dipeptides from their diastereoisomers. Accordingly, if racemization occurred at one or more residues, then, in addition to the peaks corresponding to the expected dipeptides (L-leucyl-L-amino acids) smaller satellite peaks will also appear on the recordings, demonstrating the presence of L-leucyl-D-amino acids in the mixture. The areas under the peaks allow the quantitative determination of the amount of D-amino acids in the synthetic material. There is, of course, an inherent limitation in the examination of chiral integrity of a peptide through its hydrolysis with acids, if the process of hydrolysis itself is not unequivocal in this respect. In acid hydrolysates, most amino acids appear more or less intact, but some, e.g. phenylalanine, suffer minor racemization during hydrolysis, while cystine becomes heavily contaminated with its D-isomer and also with mesocystine. Alkaline hydrolysis is even worse. It causes extensive racemization in several residues. Such details must be taken into consideration in the evaluation of the Manning-Moore analysis. This problem can be eliminated by using proteolytic enzymes for degradation.

The selectivity of proteolytic enzymes also permits their direct application for the study of optical homogeneity [41]. For instance complete digestibility of a sample with leucine amino peptidase [42, 43] provides strong evidence for the absence of D-amino acid containing peptides. A comparison of the ratios of amino acids in hydrolysates obtained on digestion of a synthetic product with proteolytic enzymes with the ratios determined in a routine acid hydrolysate is probably one of the simplest approaches for the study of chiral integrity.

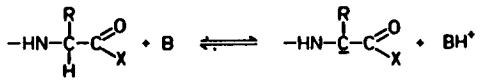
The rates of hydrolysis in degradation with proteolytic enzymes are usually low at bonds following proline and glycine residues. Some aminopeptidases, e.g. aminopeptidase M, are less restrictive in this respect. Proline, a stumbling block in proteolysis, can be set free with the help of specific prolidases [44, 45]. In addition to aminopeptidases, carboxypeptidases A, B and Y, and dipeptidylaminopeptidases can also be adopted for the same purpose. Selective cleavage, e.g. with trypsin at the carboxyl side of arginine and lysine residues, provides useful information if these were the activated amino acids of carboxyl components. In general, the stereospecificity of enzyme catalyzed hydrolysis can serve the study of optical purity in numerous ways. Perhaps less reliable is an alternative approach in which one follows the disappearance of D-amino acids from hydrolysates on treatment with D-aminoacid oxydases (e.g. from kidneys) or the elimination of L-amino acids by oxidation with

enzymes from snake venoms. The evidence obtained in these oxidative processes should be trusted only if the catalytic effect of the enzyme preparation and the conditions used are shown to be operative in control experiments with mixtures containing both L and D amino acids.

Chromatographic procedures based on columns containing chiral supports [46, 47] can differentiate between D and L amino acids. This principle, perfected by the use of high pressure liquid chromatography, might become the standard control process for the detection of racemization that occurred in the synthesis of a peptide. Reversed phase high pressure chromatography is well suited [48] also for the implementation of the Manning-Moore procedure [40] because well selected columns can completely separate the diastereoisomers formed on acylation of the amino acids in a hydrolysate with an optically pure acylating agent.

1.1.4 Conservation of Chiral Purity

Chiral purity of activated residues is affected by several factors, such as the methods of activation and protection or the nature of the activated amino acid residue. It is influenced also by the solvent used in the reaction, the presence or absence of tertiary amines, and by the basic strength and bulk of the tertiary amine if one had to be added to the coupling mixture and, last but not least, by auxiliary nucleophiles (cf. Chapter II). First and foremost of these factors seemed to be *the method of activation* and thus it received the most attention. The search for "racemization free" coupling methods is still actively pursued although this effort is fraught with an inherent difficulty. Any increase in the activation of the carboxyl group entails an increase in the acidity of the proton on the chiral α-carbon atom and facilitates, thereby, racemization via proton abstraction:

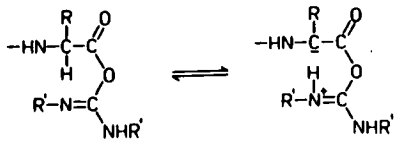


It might be more profitable to focus attention on each and every factor influencing racemization, rather than to try to develop perfect coupling methods which will yield chirally pure products under any conditions.

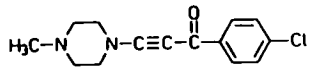
Through decades the strong belief prevailed that the azide method is free from racemization. Only later did we become aware of measurable racemization in azide coupling [21, 49, 50]. Those who observed no racemization in the preparation of peptides via azides (e.g. Ref. [27]) knowingly or intuitively avoided the use of tertiary bases, or at least did not apply tertiary amines in excess [51]. By no means do we suggest that all methods are equal in this respect. The azide method still stands out as

less conducive to racemization than many other procedures, but probably even the best methods can cause racemization under adverse conditions.

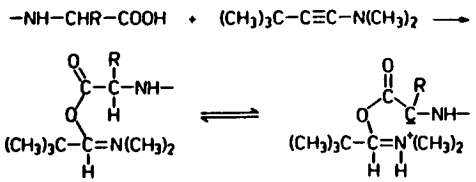
In the choice of coupling methods it is difficult to make positive recommendations, although some procedures, e.g. coupling via azides or with the help of EEDQ [52] have a fairly good record. It might be easier to point out coupling reagents which are notorious for their ability to cause racemization. Some of these, for instance the Woodward reagent [53], dicyclohexylcarbodiimide and other carbodiimides [54] caution the investigator by the structure of the reactive intermediates which contain a basic center, the potential cause of intramolecular proton abstraction from the chiral carbon atom:



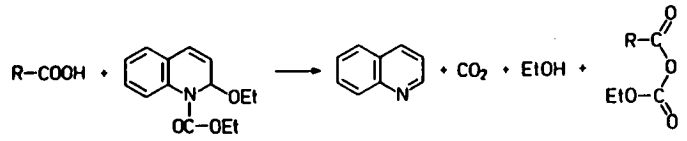
Similarly, among the various "push-pull acetylenes" [55–57] one with two basic centers [56]



is more conducive to racemization than others with only a single proton abstracting site. A basic center is generated in the earlier proposed [58] ynamines as well:

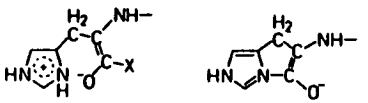


These considerations suggest that the lesser tendency of certain procedures to cause racemization is related to the absence of proton abstracting centers in the reactive intermediate and/or to the generation of materials which provide protons more readily than the chiral center of the activated residue. Thus, EEDQ [52] yields alcohol (and quinoline which has negligible basic strength):

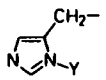


In several coupling methods substances are released which are not acidic enough to prevent acylation of the amino component, but which can, nevertheless, effectively compete with the chiral center as proton donors. This is the situation with active esters which liberate substituted phenols or hydroxylamines during coupling.

In the base catalyzed racemization of reactive intermediates the amount and concentration of the base play an obvious role. The general principle to avoid basic conditions is supported by numerous reports and hardly requires further evidence. Thus, a free amine as nucleophile is preferable to a mixture of a salt of the amino component with a tertiary base. Weak acids, e.g. 1-hydroxybenzotriazole, do not interfere with acylation and coupling can be carried out without the addition of a tertiary amine [3]. Yet, over and above the *amount of the organic base* added to the reaction mixture its *chemical character* also has significant influence on the outcome of acylation. For instance, in mixed anhydride reactions, *N*-methylmorpholine causes less racemization [49] than the widely used triethylamine. In coupling via azides 1-diethylamino-2-propanol was found to be harmless [51] while triethylamine, *N*-methylmorpholine and diisopropylethylamine had, under certain conditions, an unfavorable effect on chiral purity. The last mentioned base prevents [39] the racemization of active esters of benzyloxycarbonyl-L-phenylglycine and of *N*-benzyloxycarbonyl-*S*-benzyl-L-cysteine, but had an almost as unfavorable effect on the optical purity of benzoyl-L-leucine *p*-nitrophenyl ester as other, less hindered, tertiary amines. Apparently steric hindrance in diisopropylethylamine is insufficient to interfere with proton abstraction from azlactone intermediates. Tribenzylamine seems to be more efficient in this respect. It is quite possible, however, that the influence of bases on racemization is determined not solely by their bulkiness but also by their basic strength [59, 60]. In this connection the racemization enhancing effect of the highly nucleophilic base *p*-dimethylaminopyridine [62, 63] should also be mentioned. On the other end of the scale, the weakly basic imidazole affects unfavorably the outcome of coupling reactions, particularly if its action is intramolecular. Thus, in acylation with activated derivatives of histidine significant racemization was observed [64], presumably caused by base catalyzed enolization or by cyclization and enolization.

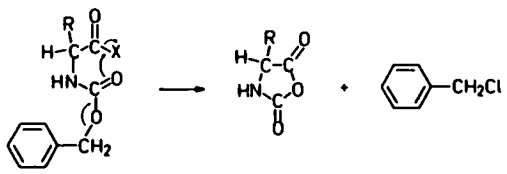


Substituents which reduce the basicity of the imidazole nucleus, e.g. the *p*-toluenesulfonyl group [65], reduce the extent of racemization as well [66]. Yet, a complete protection against loss of chiral purity of histidine residues can be expected only in derivatives in which the side chain protecting group (Y) is on the π-nitrogen atom of the imidazole:

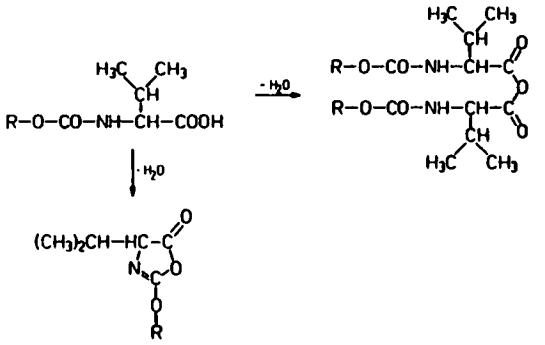


Among the factors which determine racemization the polarity of the *solvent* is quite important [4, 14]. In general, racemization is fast in highly polar solvents such as hexamethylphosphoramide, dimethylsulfoxide or dimethylformamide and is less pronounced in less polar solvents, e.g. pyridine, acetonitrile, chloroform, dichloromethane, tetrahydrofuran, dioxane or toluene. Unfortunately, most peptide intermediates are not sufficiently soluble in non-polar solvents and, at this time, the majority of acylation reactions are carried out in dimethylformamide. In solid phase peptide synthesis one applies solvents in which the peptidyl resin swells and a dissolution of the reactants is not needed. Thus, dichloromethane, which is not particularly conductive to racemization, can be used. An additional problem is created, however, by the solvent dependence of the rate of acylation of various activated intermediates. The most commonly used active esters react far better in polar solvents than in non-polar ones. These circumstances render the selection of solvents which would be favorable for acylation and yet cause little damage to chiral purity, rather difficult. A general remedy, which at least limits the extent of racemization, is to carry out the coupling reactions *at the highest possible concentration of the reactants* to ensure high coupling rates. This way the unimolecular, and hence concentration independent, racemization processes become less damaging.

A better approach to the conservation of chiral purity is offered by the *protecting groups* which are available for the blocking of the α-amino function. Already at the time of the introduction of the benzyloxycarbonyl group, its ability to protect against racemization during activation and coupling was noted and reported [67]. This unusual power to prevent the loss of chiral purity is absent from simple *N*-acyl groups such as the formyl, acetyl, trifluoroacetyl or benzoyl group and present only to some extent in the phthalyl group. On the other hand, several other amine protecting groups of the urethane type function equally well in this respect. Their ability to interfere with racemization was generally attributed to the lack of azlactone formation. The elimination of benzyl chloride and formation of *N*-carboxyanhydrides from Z-amino acid chlorides suggested [68] that

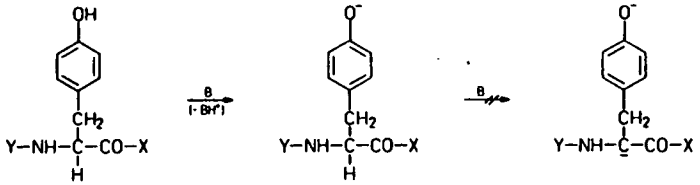


alkyloxycarbonylamino acids do not produce azlactones, the vulnerable intermediates. The formation of both the symmetrical anhydride and the 5(4*H*) oxazolone from benzyloxycarbonyl-L-valine and *tert*-butyloxycarbonyl-L-valine on reaction with water soluble carbodiimides [15] demonstrates



the imperfectness of this rationale. It seems now that, while amino acids provided with a urethane-type amine protecting group do form azlactones, the latter retain their chiral integrity even under basic conditions. Thus, the former explanation requires revision, but the empirical rule that the benzyloxycarbonyl group and other urethane-type amine blocking groups prevent the racemization of the residues to which they are attached, remains valid. Notable exceptions are the blocked derivatives of *S*-alkylcysteine, *O*-alkylserine and β-cyanoalanine. Some other amine masking groups, e.g. the *p*-toluenesulfonyl and the *o*-nitrophenylsulfenyl group, are similarly protective in this respect.

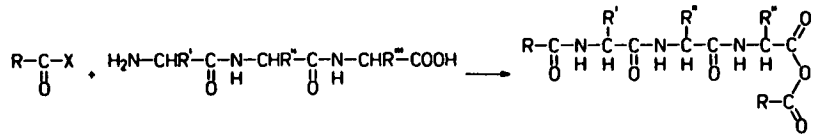
The *influence of the activated residue* on the extent of racemization can be considerable but it is not always fully understood. The benzylic character of the chiral carbon atom in phenylglycine offers a simple explanation. It is less easy to interpret the somewhat reduced chiral stability of phenylalanine moieties, probably caused by the electron withdrawing effect of the aromatic nucleus even if it is separated by a carbon atom from the chiral center. On the other hand, tyrosine with a free phenolic hydroxyl was not racemized [69] in the coupling of Z-Val-Tyr via its azide in the presence of excess base, while the azide of Z-Val-His suffered considerable loss in chiral purity under similar conditions. An explanation might be found in the abstraction of a proton from the phenolic hydroxyl: the resulting anion interferes with the abstraction of a second hydrogen and therefore the chiral carbon does not become an anionic center:



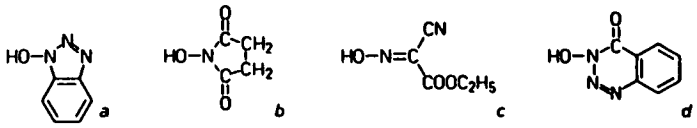
In general, formation of dianions requires stronger bases than those used in peptide synthesis.

Racemization of activated valine and isoleucine residues occurs [70] in polar solvents. The electron release by the branched side chain should destabilize the anion which has to be assumed in base catalyzed racemization processes and thus an alternative rationale must be found. The known assistance of bulky substituents in cyclization reactions might contribute to the formation of cyclic intermediates, e.g. azlactones, which play a role in the process of racemization. It is equally possible, perhaps even more likely, that, because of steric hindrance caused by bulky side chains, the coupling reactions proceed rather slowly and this allows more time for the progress of racemization. Chiral integrity is affected also by the residue(s) which precede the activated *C*-terminal amino acid in a peptide and also by the bulkiness of the *N*-terminal amino acid in the amino component [70]. The sequence dependence of racemization received, so far, only limited attention [71] and clearly requires further systematic studies.

Racemization of the *C*-terminal residue of amino components with a free *C*-terminal carboxyl was an unexpected discovery [72]. This side reaction, which is enhanced by 1-hydroxybenzotriazole and suppressed by *N*-hydroxysuccinimide, is probably due to the transient activation of the unprotected carboxyl group through interaction with the acylating agent:



One of the most powerful methods for the preservation of chiral integrity is the use of *additives* or, perhaps more appropriately, of *auxiliary nucleophiles*. These can reduce the lifetime of overactivated, racemization-prone intermediates, such as *O*-acyl-isoureas. Also, the commonly applied additives have acidic hydrogens and thus can provide a proton which is more readily abstracted by bases than the proton from a chiral center. The best results reported so far were achieved with 1-hydroxybenzotriazole [73] (a), *N*-hydroxysuccinimide [74, 75] (b), 2-hydroximinocyanoacetic acid ethyl ester [76] (c) and particularly with 3-hydroxy-3,4-dihydro-1,2,3-benzotriazine-4-one [77] (d).

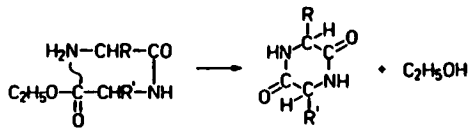


These racemization suppressing agents and several other potentially useful additives were compared by Izdebski [78].

From the foregoing discussion it is obvious that the extent of base catalyzed racemization is determined by a whole series of factors. An assessment of each of these in every coupling reaction is a demanding task and the results obtained so far are probably not entirely satisfactory since not all the influences are known, or at least not well enough to allow a quantitation of their contributions. Therefore, until the advent of truly racemization-free coupling methods, conservation of chiral integrity requires optimization in the choice of reagents, protecting groups, solvents, etc. Methods of activation which involve reactive intermediates containing a basic center should be used with caution. Overactivation, polar solvents should be avoided. The remaining choices are, however, not always conducive to an efficient formation of peptide bonds. Also, the selection of solvents is severely limited by the solubility of the intermediates. Hence, more weight has to be placed on the factors which provide some options and allow judicious decisions. For instance, the use of urethane-type amine protecting groups, attached to an amino acid rather than to a peptide, can greatly reduce the risk of racemization and the latter can be further diminished by avoiding the presence of tertiary bases in the reaction mixtures during activation and coupling. Last, but not least, the addition of well tested auxiliary nucleophiles creates conditions which no longer imperil chiral purity.

1.2 Undesired Cyclization

Dipeptide esters readily cyclize to form *diketopiperazines*. Ring closure can take place spontaneously because the thermodynamic stability of the six-membered ring overcomes the energy barrier in the formation of a *cis*-peptide bond, but the reaction is accelerated by bases, e.g. ammonia:



In solid phase peptide synthesis [79], where frequently polymer bound benzyl esters are present, this side reaction can cause some premature cleavage of the chain from the insoluble support [80–83]:

benzyloxycarbonyl groups can be carried out by catalytic hydrogenation in the presence of organic bases [240]; under the same conditions, benzyl ethers are not cleaved [241]. Peptides which provide multiple ligands for palladium, e.g. compounds with more than one methionine residue, resist hydrogenation even in the presence of base. Forced conditions, e.g. catalytic reduction for prolonged periods of time, result in desulfurization and formation of α-aminobutyric acid residues [242]. Reduction with sodium in liquid ammonia remains a viable choice, but excess sodium demethylates the methionine side chain [243].

Oxidation of the thioether to a sulfoxide occurs during the operations of peptide synthesis or during purification, but can be prevented by working in an inert atmosphere. Fortunately, oxidation to the sulfoxide is reversible. A mild treatment with thiols will reduce a sulfoxide to the thioether. Sulfones cannot be reduced under mild conditions, but they also do not form from thioethers unless powerful oxidizing agents are used.

Alkylation of the sulfur atom in the methionine side chain readily occurs during the removal of blocking groups by acidolysis [111, 119]. Some alkylations are easily reversed; e.g. *S-tert*-butyl sulfonium salts decompose on standing or on warming with the regeneration of the thioether [121]. Alkylation by the benzyl group is a more serious side reaction because *S*-benzylmethionine (salts) give rise to a variety of products [244], among them *S*-benzylhomocysteine. Therefore, in reactions where alkylating agents are generated the thioether should be kept intact with the aid of scavengers. Alternatively the methionine side chain can be protected by oxidation to the sulfoxide [123] or by reversible alkylation with methyl *p*-toluenesulfonate [124]. Alkylation by chloromethyl groups of polymeric supports should be avoided.

References

1. Bodanszky M, Martinez J (1981) Synthesis, p 333
2. Bodanszky M, Martinez J (1983) In: Gross E, Meienhofer J (eds) The Peptides, vol V. Academic, New York, p 111
3. Bodanszky M, Bednarek MA, Bodanszky A (1982) Int J Peptide Protein Res 20:387
4. Kemp DS (1979) In: Gross E, Meienhofer J (eds) The Peptides, vol I. Academic, New York, p 315
5. Iselin B, Schwyzer R, Feurer M (1955) Helv Chim Acta 38:1508; Iselin B, Schwyzer R (1960) Helv Chim Acta 43:1760
6. Kovacs J, Mayers GL, Johnson RH, Cover RE, Gatak U (1970) J Amer Chem Soc 92:1810; Kovacs J, Cortegiano H, Cover RE, Mayers GL (1971) J Am Chem Soc 93:1541
7. Barber M, Jones JH, Witty MJ (1979) J Chem Soc Perkin I 2425

8. Liberek B (1963) Tetrahedron Lett, p 1103; Liberek B, Grzonka Z (1964) Tetrahedron Lett, p 159
9. Bergmann M, Zervas L (1928) Biochem Z 203:280
10. Williams MW, Young GT (1962) J Chem Soc 27:3409
11. Neuberger A (1948) Adv Protein Chem 4:344
12. Cornforth JW (1949) In: Clarke H, Johnson JR, Robinson R (eds) The Chemistry of Penicillin, Princeton Univ, Princeton, p 800
13. Csonka FA, Nicolet BH (1932) J Biol Chem 99:213
14. Weygand F, Prox A, Schmidhammer L, König W (1963) Angew Chem 75:282
15. Jones JH, Witty MJ (1977) J Chem Soc Chem Commun 281; (1979) J Chem Soc Perkin I 3203; Benoiton NL, Chen FMF (1981) Canad J Chem 59:384
16. McDermott JR, Benoiton NL (1973) Canad J Chem 51:2562
17. Smart NA, Young GT, Williams MW (1963) J Chem Soc, p 3902
18. Williams MW, Young GT (1963) J Chem Soc, p 881
19. Anderson GW, Callahan FM (1958) J Amer Chem Soc 80:2902
20. Clayton DW, Farrington JA, Kenner GW, Turner JM (1957) J Chem Soc, p 1398
21. Weygand F, Prox A, König W (1966) Chem Ber 99:1451
22. Weygand F, Hoffmann D, Prox A (1968) Z Naturforsh 23b:279
23. Taschner E, Sokolowska T, Biernat JF, Chimiak A, Wasielewski Cz, Rzeszotarska B (1963) Liebigs Ann Chem 663:197
24. Izdebski J (1975) Roczniki Chemii 49:1097
25. Bodanszky M, Conklin LE (1967) Chem Commun, p 773
26. Spackman DH, Stein WH, Moore S (1958) Anal Chem 30:1190
27. Izumiya N, Muraoka M (1969) J Amer Chem Soc 91:2391
28. Izumiya N, Muraoka M, Aoyagi H (1971) Bull Chem Soc Jpn 44:3391
29. Benoiton NL, Kuroda K, Cheung ST, Chen FMF (1979) Canad J Biochem 57:776
30. Benoiton NL, Kuroda K (1981) Int J Peptide Protein Res 17:197
31. Halpern B, Chew LF, Weinstein B (1967) J Amer Chem Soc 89:5051
32. Benoiton NL, Kuroda K, Chen MF (1980) Int J Peptide Protein Res 15:475
33. Kitada C, Fujino M (1978) Chem Pharm Bull 26:585
34. Bosshard HR, Schechter I, Berger A (1973) Helv Chim Acta 56:717
35. Kemp DS, Wang SW, Busby G, Hugel G (1970) J Amer Chem Soc 92:1043
36. Kemp DS, Bernstein Z, Rebek J (1970) J Amer Chem Soc 92:4756
37. Kemp DS, Rebek J (1970) J Amer Chem Soc 92:5792
38. Bodanszky M, Birkhimer CA (1960) Chimia 14:368
39. Bodanszky M, Bodanszky A (1967) Chem Commun, p 591
40. Manning JM, Moore S (1968) J Biol Chem 243:5591
41. Zuber H (1968) Hoppe-Seyler's Z Physiol Chem 349:1337
42. Hill RL, Smith EL (1957) J Biol Chem 228:577
43. Hofmann K, Woolner ME, Spühler G, Schwartz ET (1958) J Amer Chem Soc 80:1486
44. Sarid S, Berger A, Katchalski E (1959) J Biol Chem 234:1740; (1962) 237:2207
45. Hill RL, Schmidt WR (1962) J Biol Chem 237:389
46. Gil-Av E, Feibush B, Charles-Sigler R (1966) Tetrahedron Lett, p 1009
47. Bayer E, Gil-Av E, König WA, Nakaparskin S, Oro J, Parr W (1970) J Amer Chem Soc 92:1738
48. Takaya T, Kishida Y, Sakakibara S (1981) J Chromatography 215:279
49. Anderson GW, Zimmermann JE, Callahan FM (1966) J Amer Chem Soc 88:1338
50. Sieber P, Riniker B, Brugger M, Kamber B, Rittel W (1970) Helv Chim Acta 53:2135
51. Kisfaludy L, Nyéki O (1972) Acta Chim Acad Sci Hung 72:75
52. Belleau B, Malek G (1968) J Amer Chem Soc 90:1651
53. Woodward RB, Olofson RA (1961) J Amer Chem Soc 83:1007; Woodward RB, Olofson RA, Mayer H (1961) ibid., 83:1010
54. Sheehan JC, Hess GP (1955) J Amer Chem Soc 77:1067

55. Gais HF (1978) Angew Chem 17:597
56. Neuenschwander M, Lienhard U, Fahrni HP, Hurni B (1978) Helv Chim Acta 61:2428
57. Neuenschwander M, Fahrni HP, Lienhard U (1978) Helv Chim Acta 61:2437
58. Buyle R, Viehe GH (1964) Angew Chem 76:572
59. Sakakibara S, Itoh M (1967) Bull Chem Soc Jpn 40:656
60. Williams AW, Young GT (1971) In: Scoffone E (ed) Peptides 1969. North Holland, Amsterdam, p 52; (1972) J Chem Soc Perkins 1, p 1194
61. Atherton E, Benoiton NL, Brown E, Sheppard RC, Williams BJ (1981) J Chem Soc Chem Commun, p 336
62. Steglich W, Höfle G (1969) Angew Chem Int ed 8:981
63. Wang SS, Kulesha ID (1975) J Org Chem 40:1227; Wang SS (1975) ibid., 40:1235
64. Windridge GC, Jorgensen EC (1971) Intra Science Chem Rep 5:375
65. Sakakibara S, Fujii T (1969) Bull Chem Soc Jpn 42:1466
66. Terada S, Kawabata A, Mitsuyasu N, Aoyagi H, Izumiya N (1978) Bull Chem Soc Jpn 51:3409
67. Bergmann M, Zervas L (1932) Ber dtsch Chem Ges 65:1192
68. Bodanszky M, Ondetti MA (1966) In: Peptide Synthesis. Wiley-Interscience, New York, p 141
69. Sieber P, Brugger M, Rittel W (1971) In: Scoffone E (ed) Peptides 1969. North Holland, Amsterdam, p 60
70. Benoiton NL, Kuroda K, Chen MF (1979) In: Siemion IZ, Kupryszewski G (eds) Peptides 1978. Wroclaw Univ, Poland, p 165
71. Weygand F, Steglich W, Boracio de la Lama X (1966) Tetrahedron Suppl 8
72. Mihara S, Takaya T, Morikawa T, Emura J, Sakakibara S In: Nakajima T (ed) Peptide Chemistry 1976, Protein Res Found, Osaka, Japan, p 36
73. König W, Geiger R (1970) Chem Ber 103:788
74. Weygand F, Hoffmann D, Wünsch E (1966) Z Naturforsch 21b:426
75. Wünsch E, Drees F (1966) Chem Ber 99:110
76. Itoh M (1973) Bull Chem Soc Jpn 46:2219
77. König W, Geiger R (1970) Chem Ber 103:2034
78. Izdebski J (1979) Polish J Chem 53:1049
79. Merrifield RB (1963) J Amer Chem Soc 85:2149
80. Lukenheimer W, Zahn H (1970) Liebigs Ann Chem 740:1
81. Gisin BF, Merrifield RB (1972) J Amer Chem Soc 94:3102
82. Khosla MC, Smeby RR, Bumpus FM (1972) J Amer Chem Soc 74:4721
83. Rothe M, Mazánek J (1974) Liebigs Ann Chem, p 439
84. Goodman M, Steuben KC (1962) J Amer Chem Soc 84:1279
85. Fruton JS, Bergmann M (1942) J Biol Chem 145:253
86. Davis NC (1956) J Biol Chem 223:935
87. Goldschmidt S, Wick M (1952) Liebigs Ann Chem 575:217
88. Wessely F, Schlögl K, Korger G (1952) Nature 169:708
89. Maclaren JA (1958) Austral J Chem 11:360
90. Bodanszky M, Sheehan JT, Ondetti MA, Lande S (1963) J Amer Chem Soc 85:991
91. Sondheimer E, Holley RW (1954) J Amer Chem Soc 76:2467
92. Battersby AR, Robinson JC (1955) J Chem Soc, p 259
93. Bajusz S, Lázár T, Paulay Z (1964) Acta Chim Acad Sci Hung 41:329
94. Schwyzer R, Iselin B, Kappeler H, Riniker B, Rittel W, Zuber H (1963) Helv Chim Acta 46:1975
95. Tam JP, Wong TW, Riemen MW, Tjoeng FS, Merrifield RB (1979) Tetrahedron Lett, p 4033
96. Ondetti MA, Deer A, Sheehan JT, Pluscec J, Kocy O (1968) Biochemistry 7:4069
97. Bodanszky M, Kwei JZ (1978) Int J Peptide Protein Res 12:69
98. Zahn H, Fölsche ET (1964) Chem Ber 102:2158

99. Meyers C, Havran RT, Schwartz IL, Walter R (1969) Chem Ind, p 136
100. Stedman RJ (1957) J Amer Chem Soc 79:4691
101. Zaoral M, Rudinger J (1959) Collect Czechoslov Chem Commun 24:1993
102. Ramachandran J, Li CH (1963) J Org Chem 28:173
103. Paul R (1963) J Org Chem 28:236
104. Bodanszky M, Fink ML, Klausner YS, Natarajan S, Tatemoto K, Yiotakis AE, Bodanszky A (1977) J Org Chem 42:149
105. Kurath P, Thomas AM (1973) Helv Chim Acta 56:1656
106. Stewart FHC (1968) Austral J Chem 21:477, 1639
107. Bodanszky M, Fagan DT (1977) Int J Pept Protein Res 10:375
108. Martinez J, Tolle JC, Bodanszky M (1979) Int J Peptide Protein Res 13:22
109. Brenner M, Curtius HC (1963) Helv Chim Acta 46:2126
110. Sakakibara S, Shimonishi Y (1965) Bull Chem Soc Jpn 38:1412
111. Weygand R, Steglich W (1959) Z Naturforsch 14b:472
112. Taschner E, Kupryszewski G (1959) Bull Acad Pol Sci Ser Chim Geol Geogr 7:871
113. Yang CC, Merrifield RB (1976) J Org Chem 41:1032
114. Bodanszky M, Martinez J (1978) J Org Chem 43:3071
115. Prestidge RL, Harding DRK, Hancock WS (1976) J Org Chem 41:2579
116. Blake J (1979) Int J Peptide Protein Res 13:418
117. Blombäck BE (1967) In: Hirs CHW (ed) Methods in Enzymology, vol 11. Academic, New York, p 398
118. Folkers K, Chang JK, Curries BL (1970) Biochem Biophys Res Commun 39:110
119. Lundt BF, Johansen NL, Vølund A, Markussen J (1978) Int J Peptide Protein Res 12:258
120. Irie H, Fujii N, Ogawa H, Yajima H, Fujino M, Shinagawa S (1976) J Chem Soc Chem Commun, p 922
121. Noble RL, Yamashiro D, Li CH (1976) J Amer Chem Soc 98:2324
122. Brenner M, Pfister RW (1951) Helv Chim Acta 34:2085
123. Iselin B (1961) Helv Chim Acta 44:61
124. Bodanszky M, Bednarek MA (1982) Int J Peptide Protein Res 20:408
125. Wünsch E, Jaeger E, Kisfaludy L, Löw M (1977) Angew Chem 89:330
126. Löw M, Kisfaludy L, Sohár P (1978) Hoppe-Seyler's Z Physiol Chem 359:1643
127. Masui Y, Chino N, Sakakibara S (1980) Bull Chem Soc Jpn 53:464
128. Löw M, Kisfaludy L, Jaeger E, Thamm P, Knof S, Wünsch E (1978) Hoppe-Selyer's Z Physiol Chem 359:1637
129. Bodanszky M, Tolle JC, Bednarek MA, Schiller PW (1981) Int J Peptide Protein Res 17:444
130. Mitchell AR, Merrifield RB (1976) J Org Chem 41:2015
131. Fujii N, Funakoshi S, Sasaki T, Yajima H (1977) Chem Pharm Bull 25:3096
132. Shin KH, Sakakibara S, Schneider W, Hess GP (1962) Biochem Biophys Res Commun 8:288
133. Sakakibara S, Shin KH, Hess GP (1962) J Amer Chem Soc 84:4921
134. Partridge SM, Davis HF (1950) Nature 165:62
135. Piszkiewicz D, Landon M, Smith EL (1970) Biochem Biophys Res Commun 40:1173
136. Brenner M (1967) In: Beyerman HC, van de Linde A, Maassen van den Brink W (eds) Peptides, Proc 8th Europ Peptide Symp. North Holland, Amsterdam, p 1
137. Khorana HG (1953) Chem Rev 53:145; cf. also Smith M, Moffatt JG, Khorana HG (1958) J Amer Chem Soc 80:6207
138. Muramatsu I, Hagitani A (1959) J Chem Soc Jpn 80:1497
139. Schnabel E (1965) In: Zervas L (ed) Proc Sixth Eur Peptide Symp Athens 1963. Pergamon, Oxford, p 71
140. Izdebski J, Kubiak T, Kunce D, Drabarek S (1978) Polish J Chem 52:539
141. Izdebski J, Kunce D, Pelka J, Drabarek S (1980) Polish J Chem 54:117
142. Izdebski J, Kunce D, Drabarek S (1980) Polish J Chem 54:413

143. Kisfaludy L, Patthy A, Löw M (1969) Acta Chim Acad Sci Hung 59:159
144. Rink H, Riniker B (1974) Helv Chim Acta 57:831
145. Honzl J, Rudinger J (1961) Collect Czech Chem Commun 26:2333
146. Schnabel E (1962) Liebigs Ann Chem 659:168
147. Bodanszky M (1955) Nature 175:685
148. Kisfaludy L, Roberts JE, Johnson RH, Mayers GL, Kovács J (1970) J Org Chem 35:3563
149. Girin SK, Shvachkin Yu P (1979) Z Obschei Khimii 49:451
150. Beaumont SM, Handford BO, Jones JH, Young GT (1965) Chem Commun, p 53
151. Handford BO, Jones JH, Young GT, Johnson TFN (1965) J Chem Soc, p 6814
152. Bodanszky M, Tolle JC (1977) Int J Peptide Protein Res 10:380
153. Wieland T, Heinke B (1956) Liebigs Ann Chem 599:70
154. Schellenberg P, Ulrich J (1959) Chem Ber 92:1276
155. Kopple KD, Renick RJ (1958) J Org Chem 23:1565
156. Zaoral M, Rudinger J (1961) Collect Czech Chem Commun 26:2316
157. Kotake H, Saito T (1966) Bull Chem Soc Jpn 39:853
158. Quitt P, Hellerbach J, Vogler K (1963) Helv Chim Acta 46:327
159. Weygand F, Steglich W, Bjarnason J, Akhtar R, Khan NM (1966) Tetrahedron Lett, p 3483
160. Stelakatos GC, Argyropoulos N (1966) Chem Commun, 271
161. Weygand F, Steglich W, Bjarnason J, Akhtar R, Chytil N (1968) Chem Ber 101:3623
162. Merrifield RB (1964) Biochemistry 3:1385
163. Savrda J (1977) J Org Chem 42:3199
164. Hoffmann K, Yajima H (1961) J Amer Chem Soc 83:2289
165. Marglin A (1972) Int J Peptide Protein Res 4:47
166. Guttmann S, Boissonnas RA (1959) Helv Chim Acta 42:1257
167. Ben Ishai D, Berger A (1952) J Org Chem 17:1564
168. Sheehan JC, Hasspacher K, Yeh YL (1959) J Amer Chem Soc 81:6086
169. Sheehan JC (1960) Ann NY Acad Sci 88:665
170. Iselin B (1962) Helv Chim Acta 45:1510
171. Spanninger PA, von Rosenberg JL (1972) J Amer Chem Soc 94:1973
172. Engelhard M, Merrifield RB (1978) J Amer Chem Soc 100:3559
173. Yajima H, Takeyama M, Kanaki J, Mitani K (1978) J Chem Soc Chem Commun, p 482
174. Nishimura O, Fujino M (1976) Chem Pharm Bull 24:1568
174a Bodanszky M, Yiotakis AE unpublished Observations
175. Schafer DJ, Young GT, Elliott DF, Wade R (1971) J Chem Soc (C), p 46
176. Windrige GC, Jorgensen EC (1971) J Amer Chem Soc 93:6318
177. Grassmann W, Wünsch E (1958) Chem Ber 91:462
178. Goodman M, Steuben KC (1959) J Org Chem 24:112
179. Grommers EP, Arens JF (1959) Rec Trav Chim Pays-Bas 78:558
180. Fontana A, Toniolo C (1976) In: Herz W, Giesbach H, Kirby GW (eds) Progress in the Chemistry of Natural Products, vol 33. Springer, New York, p 309
181. Kessler W, Iselin BM (1966) Helv Chim Acta 49:1330
182. Sieber P (1968) In: Bricas E (ed) Peptides 1968. North Holland, Amsterdam, p 236
183. Alakhov Yu B, Kiryushkin AA, Lipkin VM, Milne GWA (1970) J Chem Soc Chem Commun, p 406
184. Wünsch E, Jaeger E, Deffner M, Scharf R (1972) Hoppe-Seyler's Z Physiol Chem 353:1716
185. Previero A, Colletti-Previero MA, Cavadore JC (1967) Biochim Biophys Acta 147:453
186. Uphaus RA, Grossweiner LI, Katz JJ, Kopple KD (1959) Science 129:641
187. Previero A, Prota G, Coletti-Previero MA (1972) Biochim Biophys Acta 285:269
188. Omori Y, Matsuda Y, Aimoto S, Shimonishi Y, Yamamoto M (1976) Chem Lett, p 805

189. Hashizume K, Shimonishi Y (1980) In: Yonehara H (ed) Peptide Chem 1979. Protein Res Foundation, Osaka, Japan, p 77
190. Loffet A, Dremier C (1971) Experientia 27:1003
191. Anderson JC, Barton MA, Hardy PM, Kenner GW, McLeod JK, Preston J, Sheppard RC (1965) Acta Chim Acad Sci Hung 44:187
192. Löw M, Kisfaludy L (1979) Hoppe-Seyler's Z Physiol Chem 359:1637
193. Bajusz S, Turán A, Fauszt I, Juhász A (1973) In: Hanson H, Jakubke HD (eds) Peptides 1972. North Holland, Amsterdam, p 93
194. Schön I, Kisfaludy L (1979) Int J Pept Protein Res 14:485
195. Bodanszky M, Sigler GF, Bodanszky A (1973) J Amer Chem Soc 95:2352
196. Fraser KJ, Poulson K, Haber E (1972) Biochemistry 11:4974
197. Battersby AR, Robinson JC (1956) J Chem Soc, p 2076
198. Clayton DW, Kenner GW (1953) Chem Ind, p 1205
199. Bruckner V, Kotai A, Kovács K (1959) Acta Chim Acad Sci Hung 21:427
200. Shiba T, Kaneko T (1960) Bull Chem Soc Jpn 33:1721
201. Sano S, Kawanishi S (1975) J Amer Chem Soc 97:3480
202. Feinberg RS, Merrifield RB (1975) J Amer Chem Soc 97:3485
203. Suzuki K, Endo N, Sasaki Y (1977) Chem Pharm Bull 25:2613
204. Schwyzer R, Iselin B, Kappeler H, Riniker B, Rittel W, Zuber H (1958) Helv Chim Acta 41:1273
205. Robinson AB (1974) Proc Nat Acad Sci US 71:885
206. Riniker B, Schwyzer R (1961) Helv Chim Acta 44:685
207. Roeske R (1963) J Org Chem 28:1251
208. König W, Volk A (1977) Chem Ber 110:1
209. Sondheimer E, Semeraro RJ (1961) J Org Chem 26:1847
210. Riniker B, Brunner H, Schwyzer R (1962) Angew Chem 74:469
211. Boissonnas RA, Guttmann S, Jaquenoud PA, Waller JP (1955) Helv Chim Acta 38:1491
212. Gish DT, Katsoyannis PG, Hess GP, Stedman RJ (1956) J Amer Chem Soc 78:5954
213. Ressler C (1956) J Amer Chem Soc 78:5956
214. Stammer J (1961) J Org Chem 26:2556
215. Paul R, Kende AS (1964) J Amer Chem Soc 86:741
216. Kashelikar DV, Ressler C (1964) J Amer Chem Soc 86:2467
217. Liberek B (1961) Chem Ind, p 987
218. Wilchek R, Ariely S, Patchornik A (1968) J Org Chem 33:1258
219. Mojsov S, Mitchell AR, Merrifield RB (1980) J Org Chem 45:555
220. Bodanszky M, Denning GS Jr, du Vigneaud V (1963) Biochem Prep 10:122
221. Bodanszky M, du Vigneaud V (1959) J Amer Chem Soc 81:5688
222. Dewey RS, Barkemeyer H, Hirschmann R (1969) Chem Ind, 1632
223. Bodanszky M, Yiotakis AE unpublished
224. Jäger G, Geiger R (1970) Chem Ber 103:1727
225. Jäger G, Geiger R (1973) Justus Liebigs Ann Chem, p 1928
226. Künzi H, Manneberg M, Studer RO (1974) Helv Chim Acta 57:566
227. Mazur RH, Schlatter JM (1963) J Org Chem 28:1025
228. Fujii T, Kimura T, Sakakibara S (1976) Bull Chem Soc Jpn 49:1595
229. Sanger F (1953) Nature 171:1025
230. Benesch RE, Benesch R (1958) J Amer Chem Soc 80:1066
231. Ryle P, Sanger F (1955) Biochem J 60:535
232. Meienhofer J, Kuromizu K (1974) Tetrahedron Lett, p 3259
233. Kuromizu K, Meienhofer J (1974) J Amer Chem Soc 95:4978
234. Southard GL, Zaborowski BR, Pettee JM (1971) J Amer Chem Soc 93:3302
235. Hegedüs B (1948) Helv Chim Acta 31:737
236. Holland GF, Cohen LA (1958) J Amer Chem Soc 80:3765
237. Roeske R, Stewart FHC, Stedman RJ, du Vigneaud V (1956) J Amer Chem Soc 78:5883
238. Losse G, Stiehl HU (1981) Z Chem 21:188